



The Value of Chemokine and Chemokine Receptors in Diagnosis, Prognosis, and Immunotherapy of Hepatocellular Carcinoma

Zhengyi Wu*, Liang Sun*, Yongkang Xu *, He Huang, Zhipeng Wu, Bingbing Qiu, Jinlong Yan , Xiangbao Yin

Hepatobiliary Surgery, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi Province, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xiangbao Yin; Jinlong Yan, Hepatobiliary surgery, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, No. 1, Minde Road, Nanchang, Jiangxi Province, People's Republic of China, Email yxbefy@163.com; yjl19880608@126.com

Background: Chemokines and chemokine receptors (CCRs) are involved in a variety of anti-tumour and pro-tumour immune processes in vivo, such as angiogenesis, metastasis, proliferation and invasiveness, and influence patient prognosis and response to therapy.

Methods: CCRs differentially expressed in HCC and associated with prognosis were extracted from TCGA and GEO databases, and the obtained CCRs were then used to construct signature genes, and the signature gene were selected for expression validation as well as functional experiments to explore the role of CCRs in the treatment and prognosis of HCC.

Results: We constructed a prognostic model including five CCRs (CCL20, CCL23, CCR3, CCR10, and CXCR3) and validated the expression of signature genes. The model's risk score is an independent prognostic factor for HCC. We have also developed prognostic model nomograms for clinical use. In addition, we validated that CCR3 expression is associated with poor prognosis in HCC, and the proliferation and migration ability of HCC cells was significantly inhibited after interfering with the expression of CCR3 in MHCC-LM3. We also looked at differences in pathway enrichment, immune infiltration and immune checkpoints. Finally, we found that risk scores were also correlated with drug sensitivity, the high-risk group had a better sensitivity to sorafenib.

Conclusion: The CCRs-related gene signature may better assess HCC prognosis and response to immunotherapy and tyrosine kinase inhibitors such as sorafenib in HCC, providing prospective solutions for diagnosis and treatment.

Keywords: chemokine, chemokine receptors, prognosis, signature, hepatocellular carcinoma

Introduction

It is well known that hepatocellular carcinoma (HCC) ranks sixth in global cancer incidence and third in cancer-related deaths.¹ Most patients miss the chance for radical surgery because of the propensity to metastasize at an early stage. Surgery, local ablation, and drug-targeted therapy are currently the main HCC therapeutic options, however, the disease's mortality has not been successfully reduced.² The family of chemokines and chemokine receptors (CCRs) consists of about 50 members, most are located at 8–12 kDa, whose function is to recruit cells that then migrate to the site of inflammation or injury. Currently, chemokines are classified into 4 groups (XC, CC, CXC, CX3C).³ Chemokines have unique receptors on their target cells, more than 20 receptors have been identified.⁴ However, there are chemokines that bind multiple chemokine receptors, and similarly, some chemokine receptors have multiple ligands, suggesting that chemokines and receptors show great variation in selective binding.^{5,6} Chemokines bind to receptors to promote leukocyte migration, such as mediating the migration of monocytes, eosinophils, and dendritic cells (DCS).⁷ Chronic inflammation mediated by inflammatory cells is a long-term low-grade injury. There is a strong correlation between chronic inflammation of a specific organ and the specific cancers of that organ, such as viral hepatitis and liver cancer.⁸

When a tumour grows in the liver, some immune cells begin to surround or infiltrate the tumour tissue and abnormal aggregation occurs. CCRs play an important regulatory role in this process. Such as the CCL2-CCR2 axis is involved in the recruitment of monocytes, while it also inhibits anti-tumour immune surveillance by recruiting myeloid-derived suppressor cells.⁹ Chemokines also act directly on tumour cells and vascular endothelial cells, promoting cancer progression in the tumour microenvironment(TME).³ It has been demonstrated that in HCC, CXCL5 and CXCL8 can directly stimulate the growth of liver tumour cells as well as modulate neutrophil infiltration in the liver.¹⁰ HCC is a highly vascularised tumour. Its growth and survival depend on angiogenesis, and multiple chemokine signalling pathways are involved in this process. Tyrosine kinase inhibitors can counteract tumour angiogenesis and inhibit proliferation, and it has been demonstrated that tyrosine kinase inhibitors can target CCRs to exert anti-tumour effects.^{11,12} With the in-depth study, the biological roles of chemokines in HCC have attracted more and more attention. At present, some chemokines have been shown to have high diagnostic value in detecting HCC and liver cancer progression.¹³ However, there have been no reports on the role of chemokines and their receptors in the prediction of HCC prognosis and immunotherapy.

Materials and Methods

Data Processing

353 HCC patients and 50 normal patients data were collected from TCGA(<https://portal.gdc.cancer.gov/>) database. The data of 242 HCC patients were collected from the GEO database(GSE14520) (<https://www.ncbi.nlm.nih.gov/geo/>), and the ICGC database(ICGC-LIRI-JP) included 231 HCC patients.

Gene Screening

“limma” R package for analysis of variance (ANOVA), with FDR below 0.05 and logFCfilter above 1. Finally, 20 differentially expressed genes (DEGs) were obtained and the “limma” and “sva” R package eliminate batch effects, the expression data were also normalised and merged into a new dataset.

The new dataset contains the data of 586 HCC patients. To increase the accuracy of the COX regression results, each DEG's expression was changed to $\log_2(i+1)$. Prognostic genes were screened out using univariate COX regression analysis among DEGs.

Construct and Verification of CCRs-Related Genes Signature

The eight prognosis-related genes screened were used for signature construction. The training set (set1) and the test set (set2) are randomly divided from a total of 586 HCC samples(TCGA-LIHC and GSE14520 datasets). The merged set was used as the validation set (set 3), GSE14520 as a validation set (set 4), and the ICGC dataset as an external validation set (set 5). We perform Lasso and Cox regression analysis on the training set (set 1) to construct risk signatures. Finally, we obtain a risk-scoring system based on CCRs:

$$\text{Risk Score} = \text{mRNA expression levels} * \text{regression coefficient.}$$

Based on median risk scores, all validation sets were classified as high or low risk. We plotted ROC (1,3,5 years) and Kaplan-Meier curves to test the predictive accuracy of CCRs signature. The age, sex, stage staging, and risk score were integrated, and R packages “rms” and “replot” were used to construct nomograms based on CCRs-related genetic features. Analysis of nomogram accuracy and reliability using calibration and decision curve analysis(DCA) curve.

CCRs-Related Signature Genes Expression Validation

We used normal hepatocyte line (L02) and HCC cell lines(MHCC-97H, MHCC-LM3, HepG2, SMMC-7721 and Huh7) from the Shanghai Institute of Cell Biology. HCC and paraneoplastic tissues in ten pairs were collected after patients gave their informed consent(from the Second Affiliated Hospital of Nanchang University). Our study was conducted in accordance with the Declaration of Helsinki. All cell lines were grown in complete DMEM medium in a 5% CO₂ incubator.

RNA-level validation of gene expression: cultivate and lyse cells to obtain total RNA. The extraction process followed the instructions provided by the Trizol kit manufactured by Invitrogen. Once the total RNA was successfully extracted, and synthesis of complementary DNA (cDNA) using Takara's Reverse Transcription Kit. To measure the mRNA expression levels of key genes indicative of specific traits or conditions, qRT-PCR technique was employed.

Detection of protein expression differences using immunohistochemistry (IHC) experiments. To begin with, HCC tissues were processed and prepared for IHC analysis. The tissue samples were first embedded in paraffin. It is then cut into thin slices, ensuring a precise and consistent thickness. Before IHC staining, the slices need to be dewaxed and hydrated. After preparing tissue slices, specific targeting antibody and secondary antibody incubations were performed sequentially. After the appropriate incubation time, the sections were subjected to a staining procedure and photographed.

siRNAs and Transfection

The CCR3 siRNA and control siRNA was obtained from General Biology Co., Ltd. Lipofectamine 3000 (Invitrogen) for cellular transfection of siRNAs and the efficiency of the intervention was tested by Western Blot and transfected cells were used in subsequent experiments.

Cell Proliferation Assay

We used the EdU assay to determine proliferation efficiency by first inoculating cells into 96-well plates (1*10⁴/well) and incubating for 24 hours. Staining was performed by adding 100 μ L EDU (UELANDY) to well, followed by fixation with paraformaldehyde and neutralisation with 2 mg/mL glycine solution after 20 min. Next, it was washed with PBS, and then 1 \times The Apollo Stain was added for staining, and after 30 min it was washed with 0.5% TritonX-100 solution. Stain with 1 \times Hoechst 33,342 for 20 minutes, then wash with PBS and the positive cells were observed by fluorescence microscopy.

Transwell Assay

First, 200 μ L of serum-free medium was added to the upper chamber and 600 μ L of complete medium was added to the lower chamber, and the cells (1.5*10⁴/well) were inoculated into the upper chamber and cultured. After a period of incubation, it was fixed with paraformaldehyde and then stained with crystal violet. The migrated cells were then counted using a microscope.

Wound Healing Assay

Cells were first inoculated in a 24-well plate, and linear wounds were made with 200- μ L pipette tip after the cells had adhered to the wall. Images of the wound closure were then recorded at 0 hours, 24 hours and 48 hours. The area of scratch was determined using Image J software.

Functional Enrichment Analysis

Functional enrichment of CCRs-related gene models was performed by GSEA, including KEGG and GO enrichment analysis between risk groups, the images show the five most significant enrichment pathways.

Tumor Immune Infiltration and Microenvironment

The CIBERSORT algorithm to assess the immune cell content, and then violin plot demonstrates the difference in immune cells in the risk group. The “ggpubr” R package was used to analyse the tumour microenvironment (TME) and microsatellite instability (MSI), thus validating the link between risk features and TME as well as immunotherapy.

Immunotherapy and Drug Sensitivity

The differential expression of immune checkpoints was determined using the “ggpubr” R package, along with analyse how immune checkpoints correlated with signature genes. The feasibility of immunotherapy based on tumour immune dysfunction and exclusion (TIDE). In addition, changes in sorafenib IC₅₀ between risk groups and association with risk scores were examined using the “prophetic” R package.

Statistical Analysis

R language (version 4.1.2) and graphpad prism 8.0 were used for statistical analysis, and the two groups were analysed using the *t*-test, with $P < 0.05$ indicating significance.

Results

Screening for CCRs-Related Differential and Prognostic Genes

Research shows that a total of 65 CCRs related genes have been discovered so far (Table S1). The expression data of 65 CCRs in TCGA-LIHC were extracted and analyzed, and 20 DEGs were obtained (14 high expression, 6 low expression) (Figure 1A and B, Table S2). First, enrichment analysis of the DEGs obtained, and GO enrichment analysis suggested that they act in chemokine-mediated signalling pathways (BP), plasma membrane outer (CC) and chemokine activity

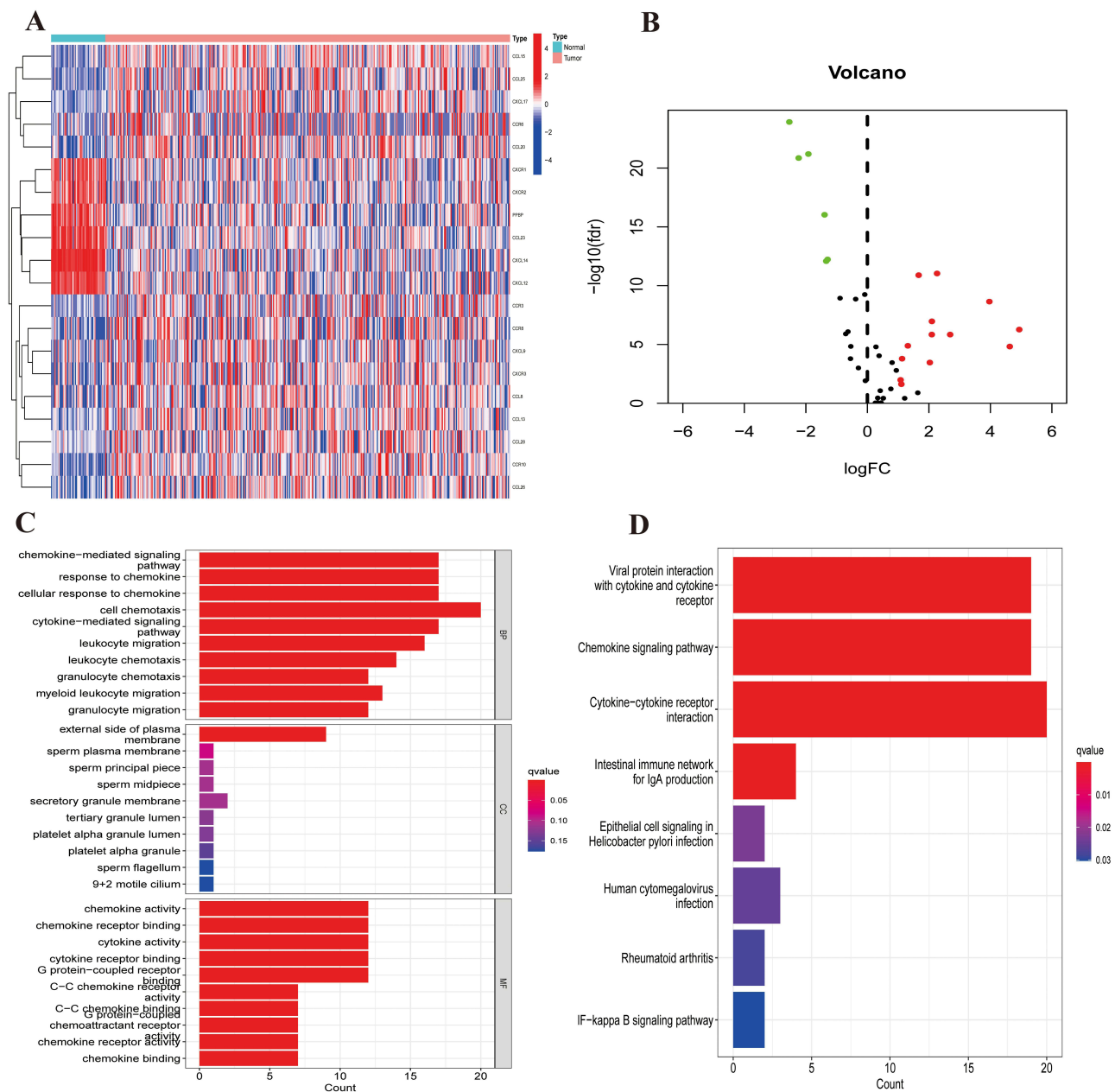


Figure 1 Identification of differentially expressed genes (DEGs) in HCC. **(A)** The heatmap. **(B)** The volcano map. **(C)** Gene ontology (GO) analysis of DEGs. **(D)** Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of DEGs.

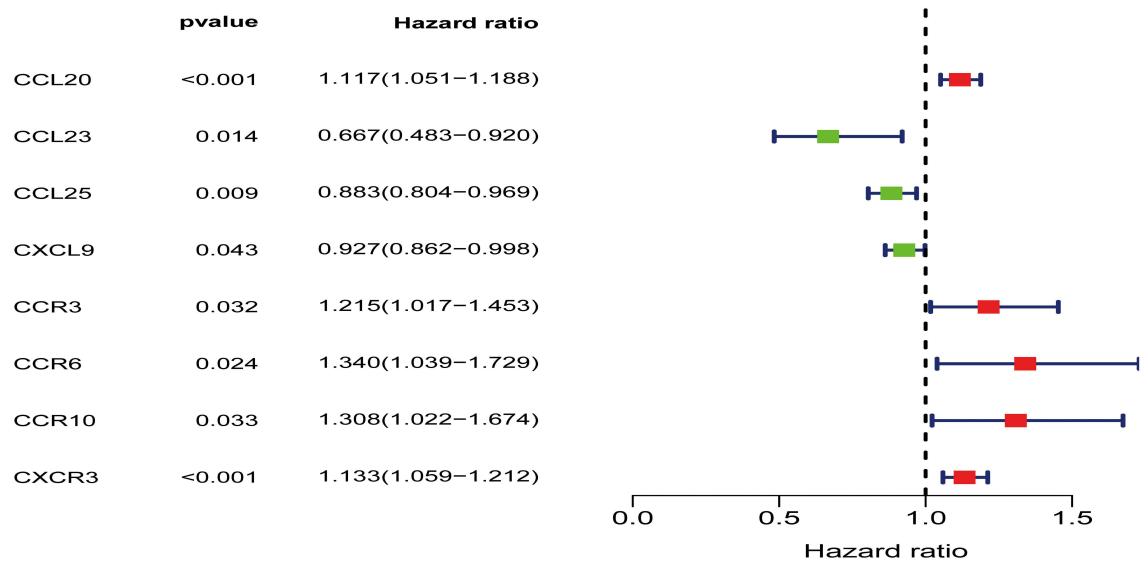
(MF). KEGG enrichment analyses suggested a major association with viral protein interaction with cytokine and cytokine receptors, Chemokine signaling pathway, etc (Figure 1C and D). In addition, we combined expression data and survival data of 586 HCC patients, excluding patients with less than 31 days of survival, and finally screened out 8 genes of the DEGs that were significantly associated with prognosis (Table S3).

CCRs-Related Gene Signature Construction

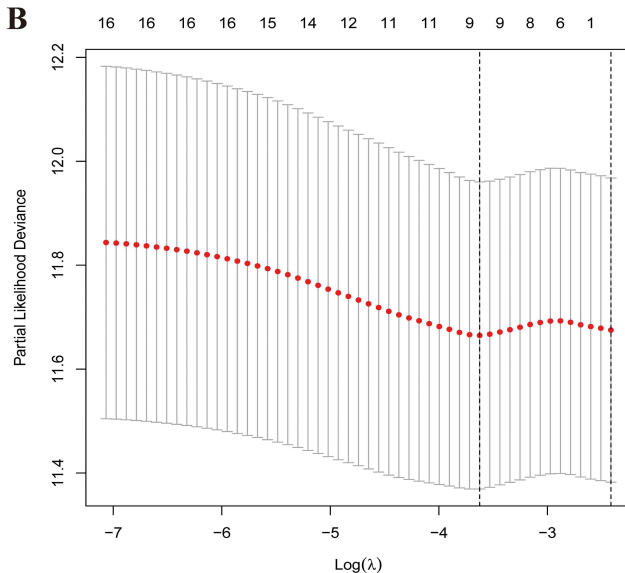
We obtained signature genes based on these 8 genes through Lasso and multivariate COX regression analysis. When the penalty index is 9, the model fits best. Finally, the model consists of 5 signature genes: CCL20, CCL23, CCR3, CCR10, and CXCR3 (Figure 2A–C). In combination with the characteristic genes and their regression coefficients, we created a risk scoring system, the formula is as follows:

$$\text{RiskScore} = (\text{CCL20} * 0.083) - (\text{CCL23} * 0.367) + (\text{CCR3} * 0.323) + (\text{CCR10} * 0.720) - (\text{CXCR3} * 0.396)$$

A



B



C

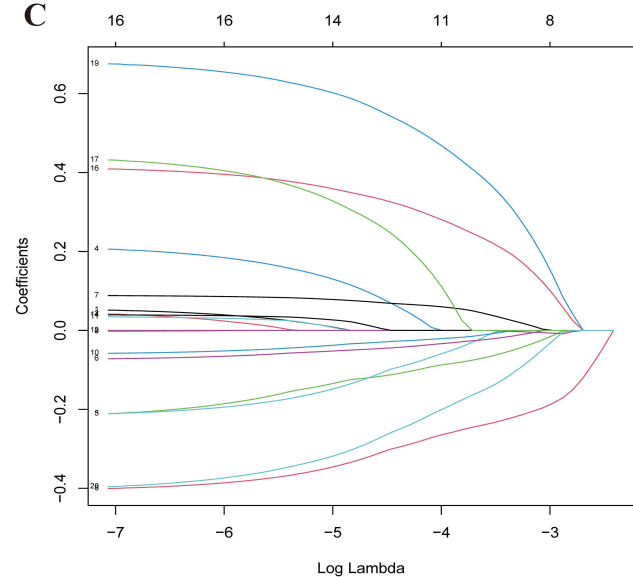


Figure 2 COX and LASSO regression analysis to identify signature genes in the training set. (A) We used univariate cox regression analysis to screen prognostic related genes in the training set. (B) Cross-validation of the LASSO regression. (C) Coefficient value of prognostic genes.

We mapped the differential expression heatmap of the 5 CCRs-related signature genes in the training set (set1) (Figure 3A). We found increasingly shorter survival times and more patients dying as the risk score increased (Figure 3B). In the set 1, survival rate was lower in the high-risk group ($P < 0.01$) (Figure 3C). The AUC at 1, 3 and 5 years were 0.643, 0.671 and 0.638 respectively (Figure 3D).

Validation of Expression of CCRs-Related Signature Genes

RT-qPCR for detection of mRNA expression levels of signature genes in cells and tissues (Figure 4A), and the sequences of the primers are shown in Table S4. The results showed that the expression of CCL20, CCR3, CCR10 and CXCR3 in HCC cells was significantly higher than that in normal liver cells. In contrast, the expression of CCL23 was higher in normal liver cell. In addition, we examined the mRNA expression of the signature genes in 10 pairs of HCC tissues and their paraneoplastic tissues (Figure 4B). We demonstrated representative images of signature genes through immunohistochemical experiments. CCL20, CCR3, CCR10, and CXCR3 are significantly overexpressed in HCC tissues, while CCL23 is significantly overexpressed in paraneoplastic tissues (Figure 5).

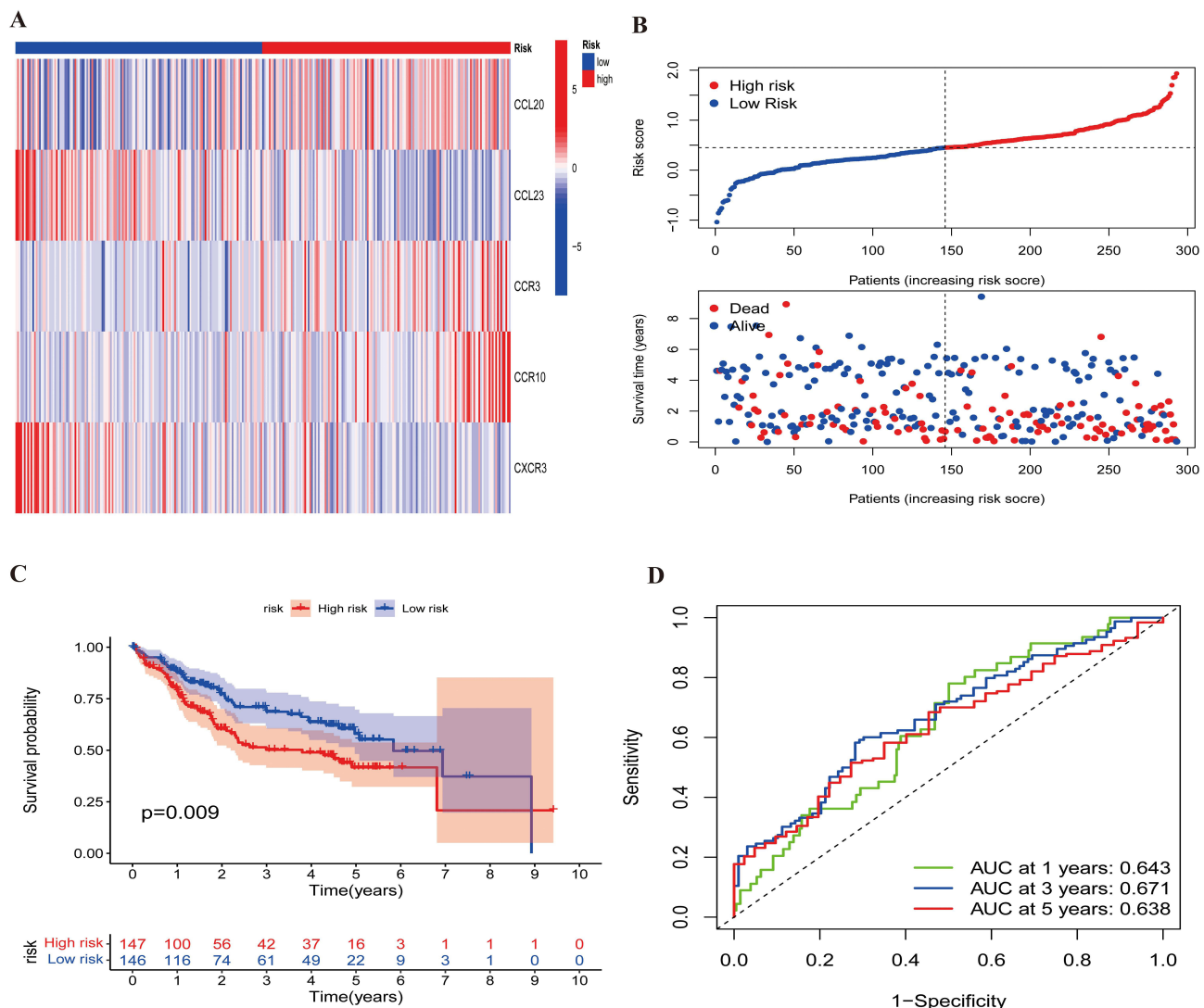


Figure 3 Prognostic signature based on 5 CCRs-related genes. (A) The expression of 5 signature genes in the train set. (B) Survival analysis between the high-risk and low-risk groups in the train set. (C) Distribution of risk scores and survival outcomes. (D) Receiver operating characteristic curve (ROC) of risk score in the train set.

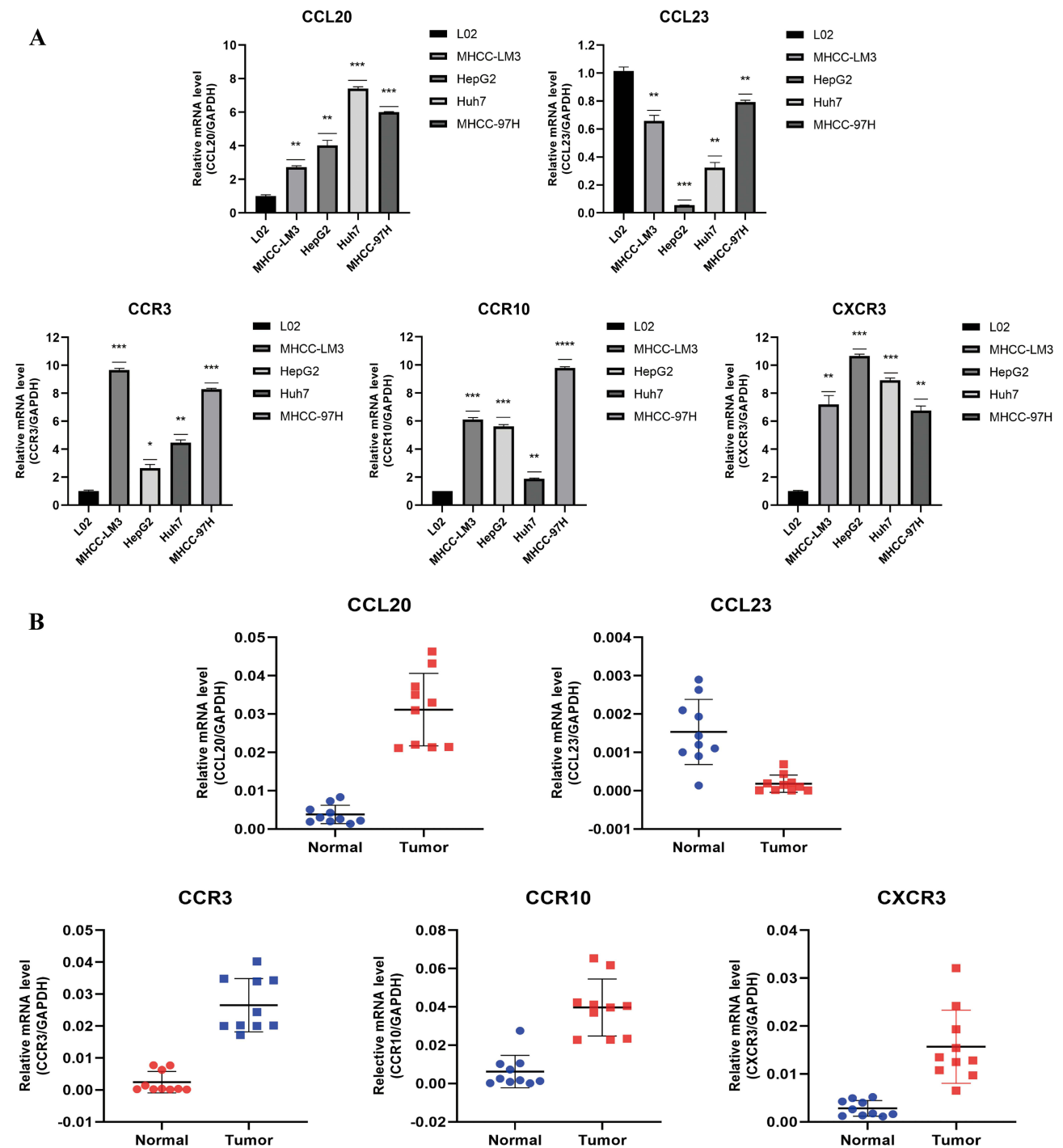


Figure 4 Validation of differential expression of 5 signature genes in cells and tissues by RT-qPCR. **(A)** Differential mRNA expression of 5 signature genes in L02, MHCC-LM3, HepG2, Huh7 and MHCC-97H cells. **(B)** Differential mRNA expression of 5 signature genes in HCC tissue and paraneoplastic tissue (10 pairs).

CCRs-Related Gene Signature Validation

We obtained genes with possible interactions with CCRs-related signature genes from the online GeneMANIA website (<http://genemania.org/>). Finally, We found that their function were mainly related to cellular response to chemokine, cytokine receptor binding, etc ([Figure S1](#)).

In the test set (set 2), validation set 3 (set 3), validation set 4 (set 4) and set 5, there were significant differences in survival rates between risk groups, with lower survival rates in the high-risk group. The AUC values indicated that the

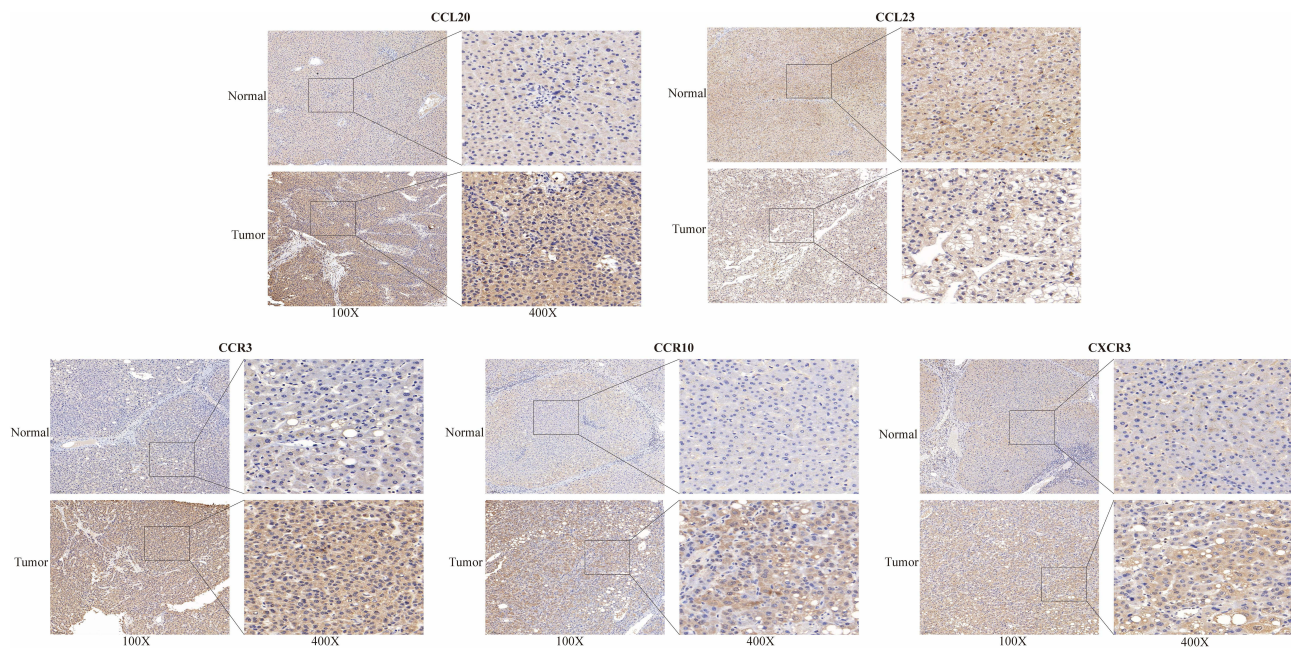


Figure 5 Staining images of five signature genes in HCC tissues and paraneoplastic tissues.

model had a certain degree of reliability (Figure S2). We also tested the degree of classification by t-SNE and PCA downscaling in set 3 and set 5, and patients in the high-risk and low-risk groups could be well discriminated (Figure S3).

Independent Prognostic Analysis

We combined risk scores and clinical data to assess the independent prognostic power of the model. And found that stage and risk score were prognostic factors for patients in univariate and multivariate analysis ($p < 0.001$) (Figure 6A and B). For the model to apply to individual patients, we constructed a nomogram of the prediction model (Figure 6C). The nomogram has good predictive ability, as shown by calibrated plots of 1, 3 and 5 year (Figure 6D). For the DCA curve, the nomogram also has a better predictive value than other clinical data (Figure 6E).

Poor Prognosis in HCC Associated with CCR3 Expression

CCL20, CCL23, CCR10, CXCR3 have been reported to mediate the progression and prognosis of HCC. Therefore, we chose CCR3, a signature gene that has not been studied before, for our HCC prognosis experiments. First we verified the correlation between CCR3 expression and survival time in two independent datasets, TCGA and GSE14250, with patients with high CCR3 expression having shorter survival times (Figure 7A and B). Next, we looked at CCR3 expression in cells and tissues, and CCR3 expression was significantly higher in HCC cell lines and HCC tissues (Figure 7C–E). We then used the MHCC-LM3 cell line with the highest expression level of CCR3 for subsequent experiments. We verified the intervening efficiency by Western Blot (Figure 7F and G).

To evaluate how CCR3 expression affects the growth of HCC cells. In the MHCC-LM3 cell line, we carried out tests with the EdU assay. The rate of cell proliferation in the experimental group (si-CCR3) was then discovered to be much lower than the control group (si-NC) following interference with the expression of CCR3 in MHCC-LM3 (Figure 8A and B). The transwell and wound healing assay revealed that HCC cells' capacity to migrate was considerably reduced after CCR3 expression was suppressed (Figure 8C–F).

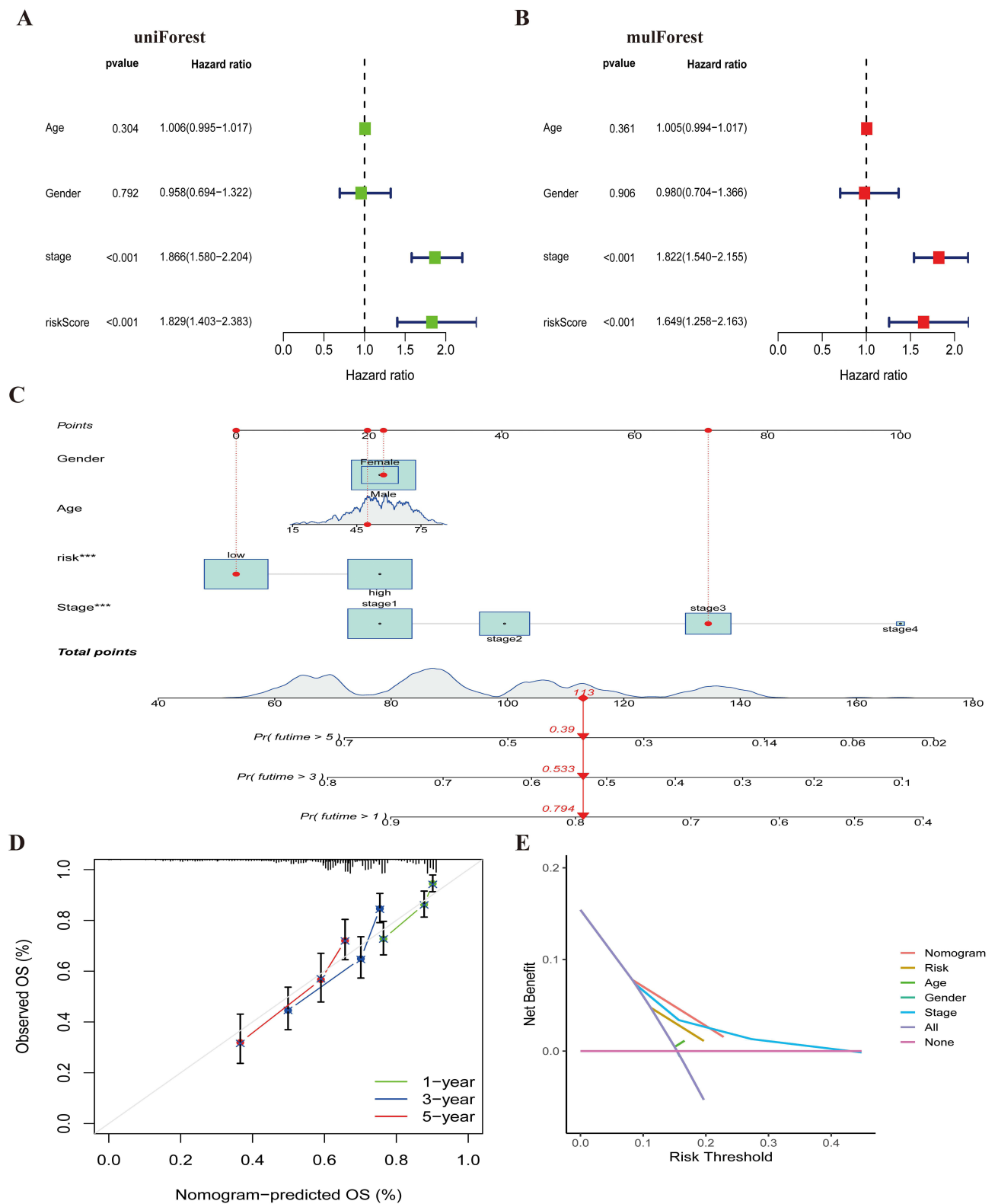


Figure 6 Independent prognostic analysis and Construction and validation of the Nomograms. **(A)** Univariate cox regression analysis. **(B)** Multivariate cox regression analysis. **(C)** Construction of the Nomograms. **(D)** The calibration curves displayed the accuracy of the nomogram in the 1, 3 and 5 years. **(E)** The decision curve analysis (DCA).

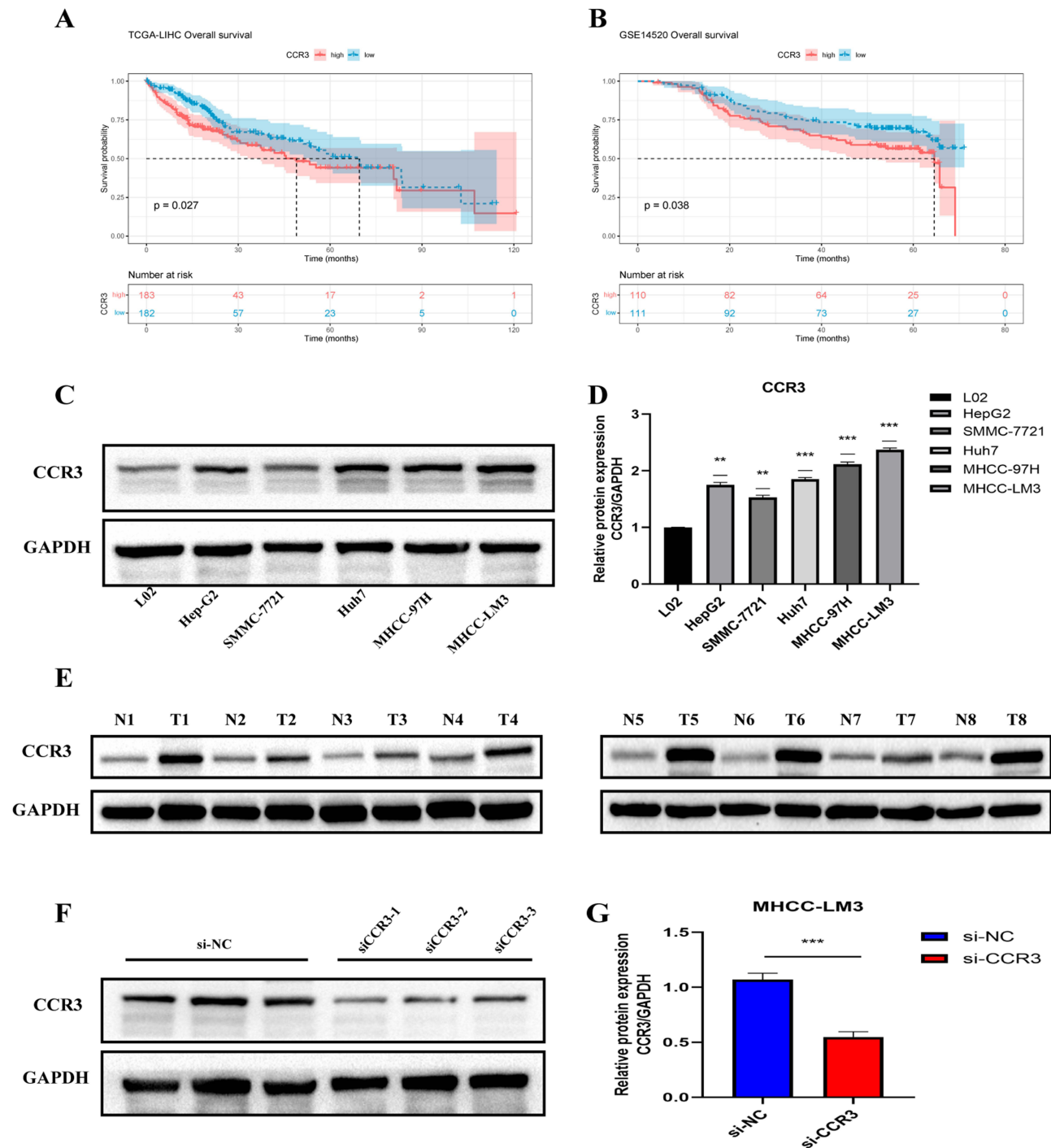


Figure 7 The expression level of CCR3 in HCC cell lines and tissues, and then verify the intervention efficiency. **(A and B)** Survival differences between high and low CCR3 expression groups in the TCGA and GSE14520 datasets. **(C and D)** the expression level of CCR3 in HCC cell lines(HepG2, SMMC-7721, Huh7, MHCC-97H, MHCC-LM3) is significantly higher than that in normal liver cell lines(L02), and the expression level is highest in MHCC-LM3. **(E)** Differential protein expression of CCR3 in 8 pairs of HCC tissues and paraneoplastic tissues. **(F and G)** Verifying intervention efficiency through Western Blot, after intervening with the expression of CCR3, the expression in MHCC-LM3 was significantly lower than that in the control group. ** $P < 0.01$, *** $P < 0.001$.

Clinical Correlation Analysis

We analysed differences in survival by clinical stage and age. The results suggest that survival is lower in high-risk groups at different stages and ages ($p < 0.05$) (Figure 9A–D). In addition, The difference between clinical features and risk scores is shown in the box plots (Figure S4).

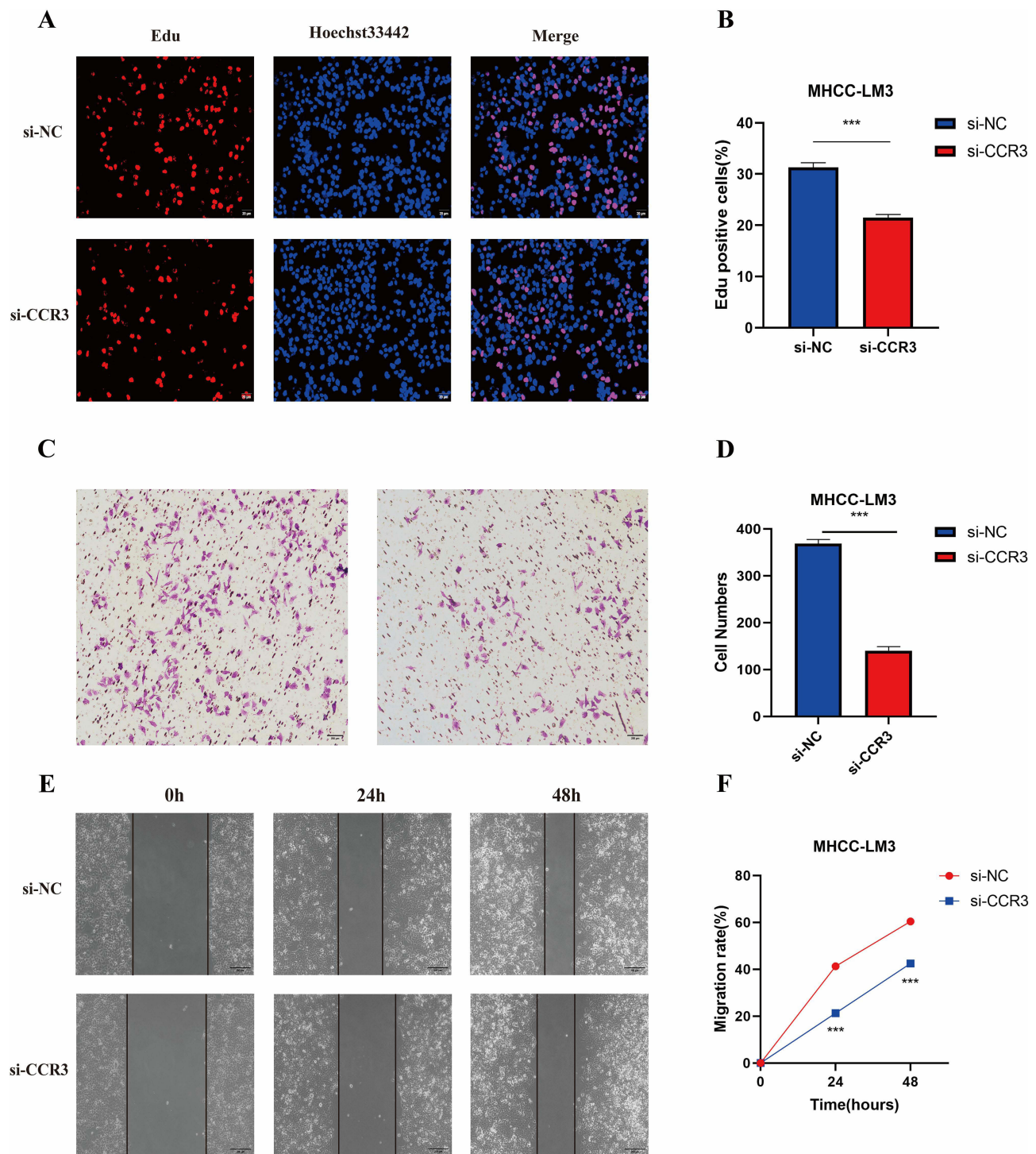


Figure 8 Verifying the correlation between the expression of CCR3 and the poor prognosis of HCC. (**A** and **B**) EdU assay: the cell proliferation rate of si-CCR3 group was significantly lower than that of si-NC group. (**C** and **D**) Transwell assay: after intervening with the expression of CCR3, the ability of cell migration was significantly lower than that of the control group. (**E** and **F**) Wound healing assay: the migration ability of HCC cells were significantly inhibited after the intervention of BSG expression. *** $P < 0.001$.

GO and KEGG Enrichment Analysis

We analysed functional differences between two risk groups using GO and KEGG. GO enrichment analysis revealed: The high-risk group is enriched in GOBP_CHROMOSOME_SEGREGATION, GOBP_MITOTIC_SISTER_CHROMATID_SEGREGATION, GOBP_NUCLEAR_CHROMOSOME_SEGREGATION, GOBP_SISTER_CHROMAT

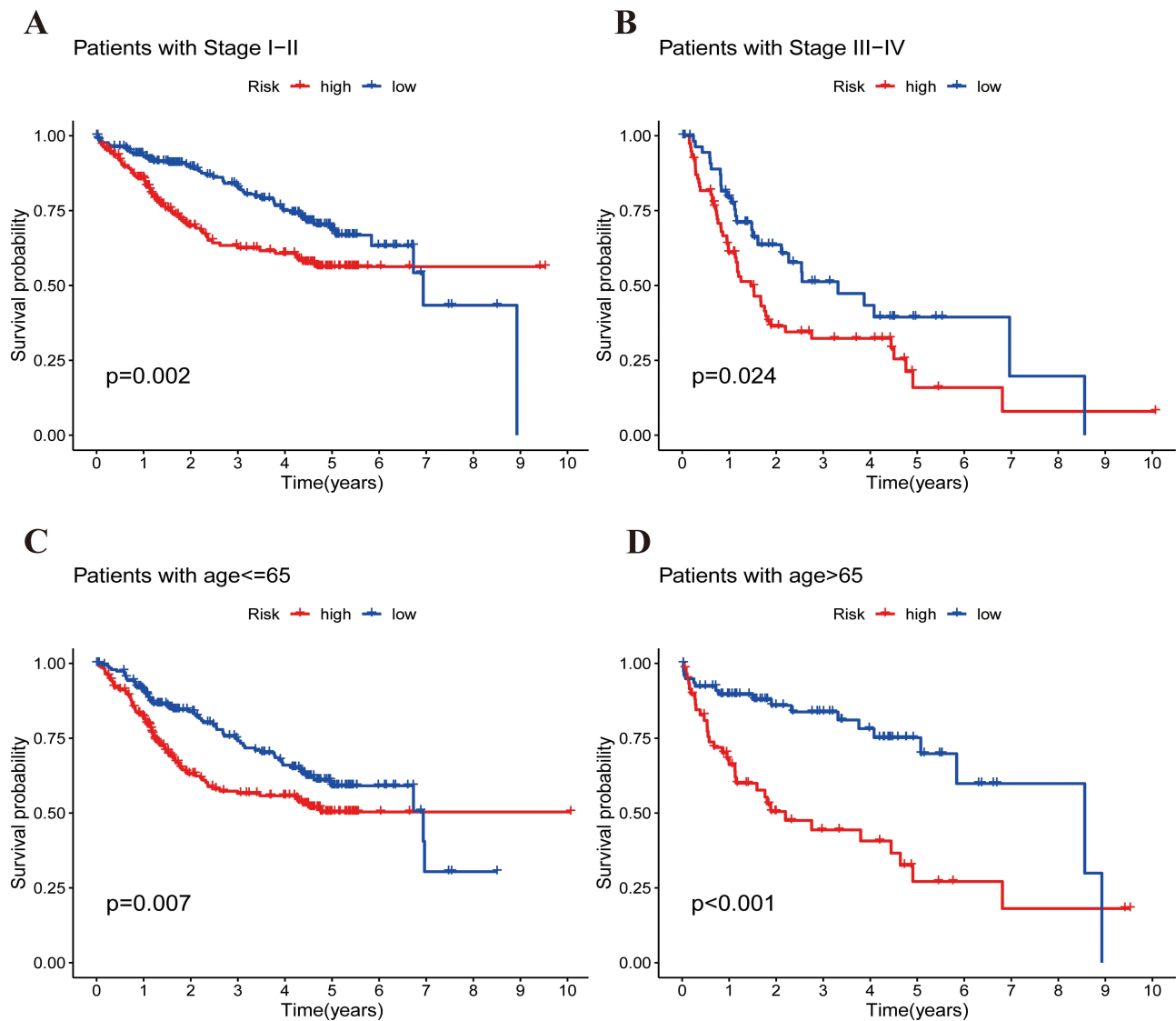


Figure 9 Relationship between risk score and clinical characteristics. **(A)** Survival analysis between the high-risk and low-risk groups in the patients with Stage I-II. **(B)** Survival analysis between the high-risk and low-risk groups in the patients with Stage III-IV. **(C)** Survival analysis between the high-risk and low-risk groups in the patients younger than 65 years old. **(D)** Survival analysis between the high-risk and low-risk groups in the patients over 65 years old.

ID_SEGREGATION and GOCC_CHROMOSOMAL_REGION. The low-risk group is enriched in GOBP_ACTIVATION_OF_IMMUNE_RESPONSE, GOBP_ADAPTIVE_IMMUNE_RESPONSE, GOBP_B_CELL_RECEPTOR_SIGNALING_PATHWAY, GOBP_T_CELL_SELECTION and GOMF_ANTIGEN_BINDING (Figure 10A and B). KEGG analysis suggested: The high-risk group is enriched in KEGG_CELL_CYCLE, KEGG_DNA_REPLICATION, KEGG_PYRIMIDINE_METABOLISM, KEGG_RIBOSOME, and KEGG_SPLICEOSOME. The low-risk group is enriched in KEGG_CELL_ADHESION_MOLECULES_CAMS, KEGG_COMPLEMENT_AND_COAGULATION_CASCADES, KEGG_DRUG_METABOLISM_CYTOCHROME_P450, KEGG_GRAFT_VERSUS_HOST_DISEASE, KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS (Figure 10C and D).

Immune Infiltration Analysis and TME

To explore how prognostic models relate to immune cell infiltration, We used violin plots to show the infiltration fraction of immune cells. High-risk group had more macrophages M0 and neutrophils infiltration ($p<0.05$), and T cells CD8 and gamma delta are more infiltrated in the low-risk group ($p<0.05$) (Figure 11A). We also analysed

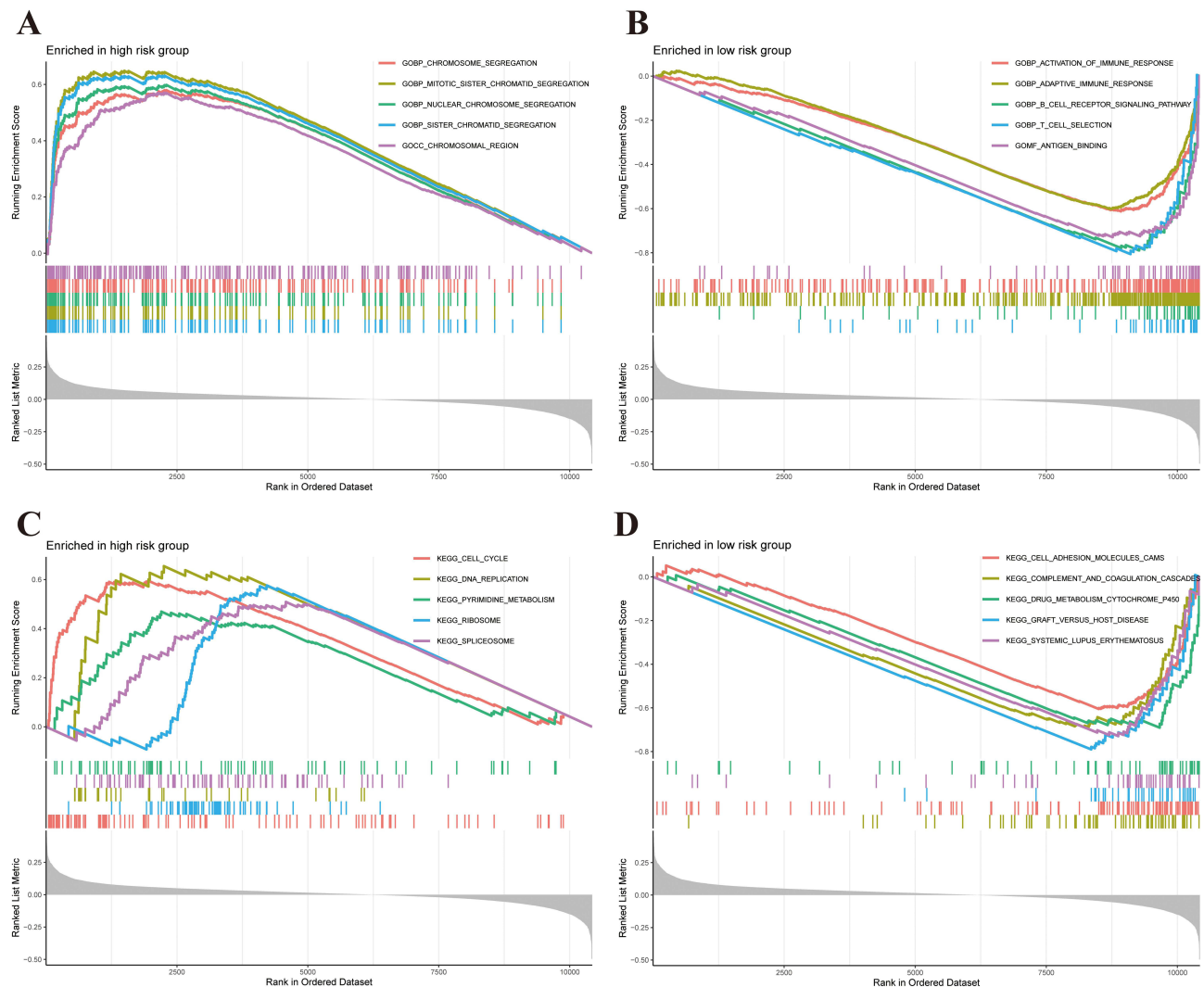


Figure 10 GO and KEGG enrichment analysis of CCRs-related genes. (A) GO enrichment analysis of high-risk group. (B) GO enrichment analysis of low-risk group. (C) KEGG enrichment analysis of high-risk group. (D) KEGG enrichment analysis of low-risk group.

the differences of immune-related functions, there were significant differences in 12 immune-related functions ($p < 0.05$) (Figure 11B). In the TME score, the low-risk group had better stromalscore, immunescore and estimate-score (Figure 11C), indicating lower tumour purity. MSI levels were also lower in the low-risk group ($P < 0.05$) (Figure 11D). We have summarised the relationship between signature genes and immune cells (Figure S5).

Analysis of Immune Checkpoints and Drug Sensitivity

Among the differences in immune checkpoint expression, TNFRSF9, LAG3, CTLA4, ICOS, CD40, CD28 and CD80 were higher in the low-risk group (Figure 12A). There were mostly positive correlations between the signature gene and immune checkpoints (Figure 12B). TIDE showed that the high-risk group may have better immunotherapy outcomes due to their lower scores (Figure 12C). In addition, cancer stem cells are involved in several tumour processes, so we analysed their relationship and found a positive correlation ($P < 0.05$) (Figure 12D). When we analysed drug sensitivity, we found that patients with higher risk scores were more sensitive to sorafenib and may have better treatment outcomes (Figure 12E and F).

Discussion

CCRs are small secreted cytokines that play a role in inflammatory and immune processes by inducing the migration of immune cells throughout the body.^{14,15} They affect the changes in TME by regulating inflammatory and immune

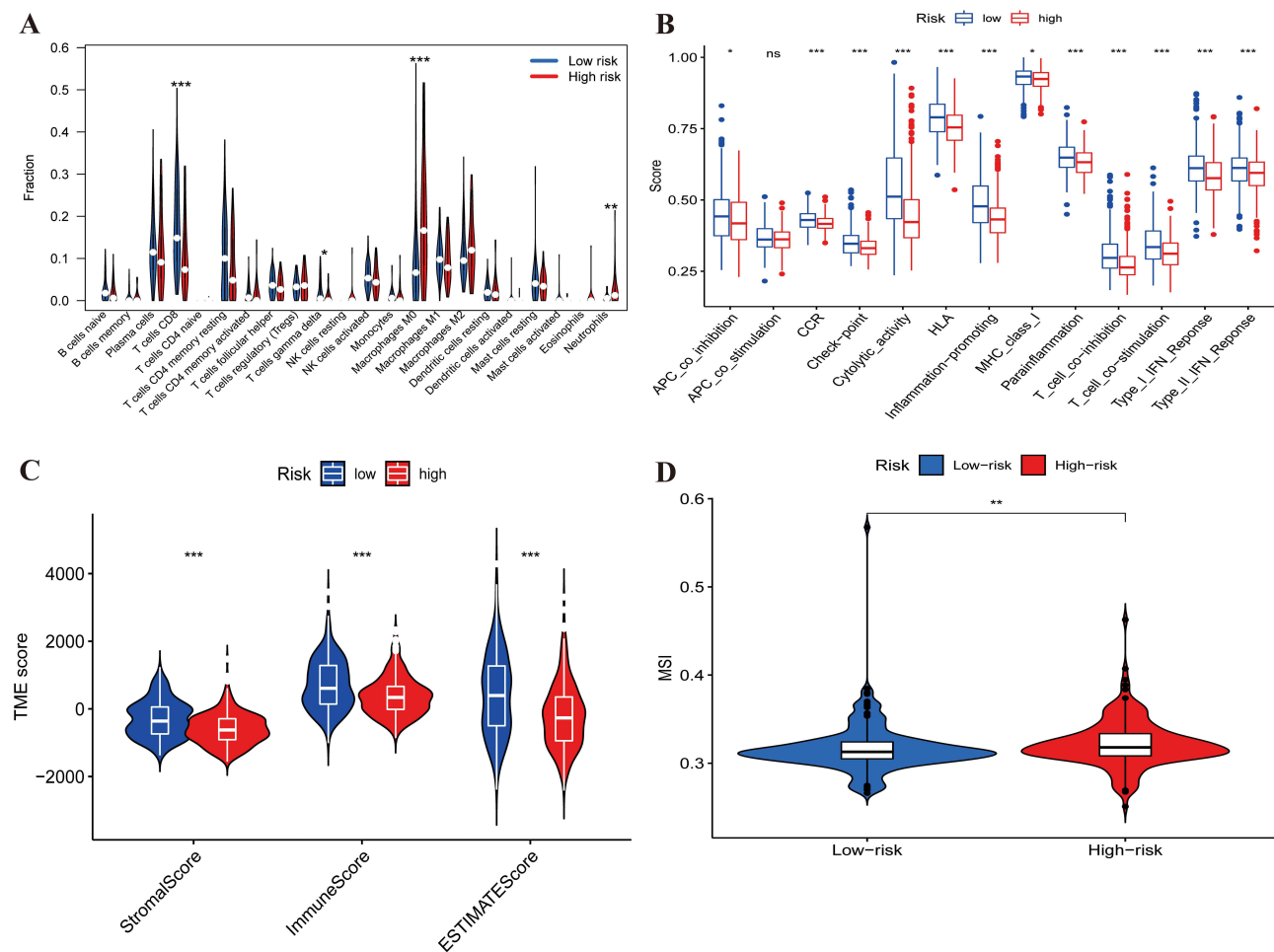


Figure 11 Immune infiltration analysis and Analysis of the Tumor microenvironment (TME) and Microsatellite instability (MSI). **(A)** The comparison of CIBERSORT scores derived from 22 different immune cells. **(B)** Analysis of immune-related pathways between high-risk group and low-risk group. **(C)** The TME score levels between high-risk and low-risk group. **(D)** The expression of MSI between high-risk group and low-risk group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

responses in the body, and participate in various pro-tumor and anti-tumor immune processes.¹⁶ Existing research have highlighted the critical role of the TME in the progression of HCC, in particular the role of the TME in regulating the progression of liver fibrosis, the development of HCC, the onset of epithelial-mesenchymal transition (EMT).¹⁷ Our study establish a signature based on CCRs-related genes in HCC, providing a new analytical marker and immune and therapeutic predictive factor, providing a new approach for individualized treatment of HCC.

Our signature established by the five CCRs(CCL20, CCL23, CCR3, CCR10, CXCR3). Through validation of this model, we found that it can be an accurate predictor of the prognosis of HCC patients. This model is a standalone predictive factor for HCC patients, unlike conventional clinical and pathological features. We also validated the expression of signature gene in cells and tissues. This signature can all serve as predictive factor for HCC patients.

For these 5 signature genes, CCL20 and its receptor CCR6 have been shown to play a role in tumour proliferation and metastasis.^{18,19} In HCC, they induce HCC cell proliferation, adhesion, and chemotactic migration. At the same time, they are also driving factors for tumour angiogenesis in HCV-related HCC, and active angiogenesis in HCC is not only associated with tumour growth but also contributes to its metastatic potential.^{20–22} CCL23 is a relatively young chemokine. In the study of acute myeloid leukemia, ischemic stroke, coronary atherosclerosis, and other diseases, the expression of CCL23 has been proven to be related to the disease process.^{23,24} Recent research in HCC has shown that CCL23 can boost anti-tumour immunity by reducing Endoplasmic reticulum stress and helping to recruit macrophages and dendritic cells²⁵(Karan, 2021). In addition, CCL23/TFAP4 may inhibit angiogenesis, thereby achieving the process of inhibiting HCC. CCR3 is the most important receptor for chemokines CCL11, CCL24, and CCL26, mainly expressed in vascular endothelial cells. After binding

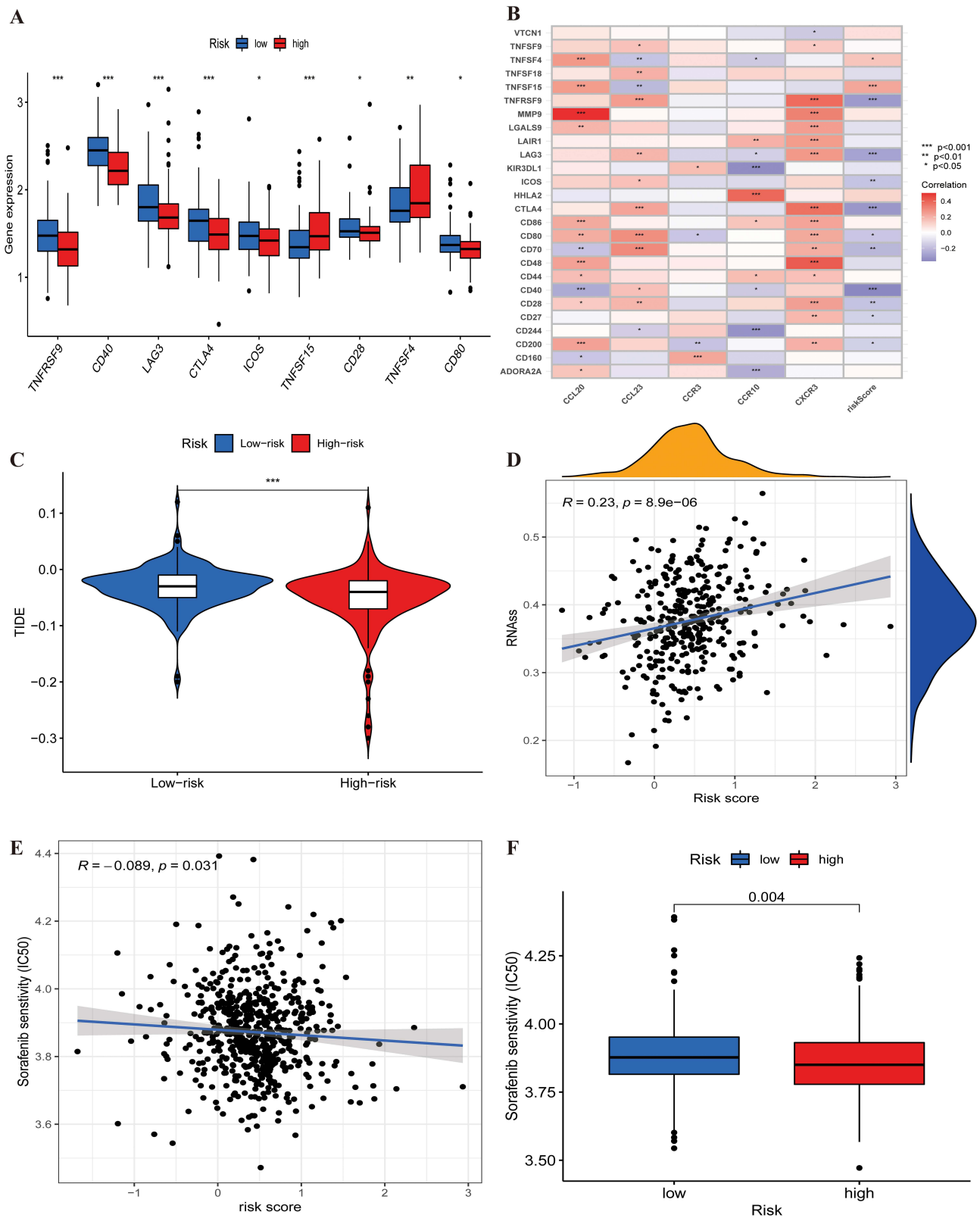


Figure 12 Evaluation of immune checkpoint profiles and immunotherapy between risk groups. **(A)** The expression of immune checkpoints between high-risk group and low-risk group. **(B)** Correlation analysis between immune checkpoints and risk scores. **(C)** Comparison of the scores of TIDE between the high and low risk group. **(D)** The relationship between risk score and RNAs. **(E)** The relationship between risk score and sorafenib sensitivity (IC50). **(F)** Comparison of the sorafenib sensitivity (IC50) between the high and low risk group.

with CCR3, the main function is to promote angiogenesis, thereby mediating tumor progression.^{26,27} However, it is not known whether CCR3 is associated with HCC progression. We therefore chose CCR3 to study how it relates to HCC progression. We found that poor prognosis in HCC associated with CCR3 expression, and high CCR3 expression may encourage HCC cells' capacity to proliferate and migrate. CCR10 is the receptor of chemokines CCL27 and CCL28. CCL27 is mainly derived from keratinocytes and therefore acts primarily in the skin,²⁸ while CCL28 is produced in mucosal tissue.²⁹ When CCR10 binds to CCL27 and CCL28, it induces the aggregation of immune cells towards epithelial cells. Because different types of lymphocytes are recruited, they play opposite roles in tumour development. When they recruit helper T cells, cytotoxic T cells and natural killer cells, they inhibit tumour progression. Conversely, if they recruit regulatory T cells or myeloid inhibitory cells, they promote tumour progression.³⁰ Studies have shown that the CCR10-CCL28 axis can promote HCC migration and invasion under hypoxic conditions.³¹ Activated T cells, B cells, and natural killer cells are the main cell types that express CXCR3. Upon binding to ligands, it induces migration of immune cells, thereby mediating infection, auto-immunity and tumour immunity. In the study of HCC, CXCR3 promotes HCC migration and invasion by binding to CXCL9 and then activating the p-ERK1/2-MMP2/MMP9 signalling pathway.³²

Tumour immunotherapy is a clinically proven treatment that works by enhancing the function of the immune system to control the growth and spread of tumours. A number of immunotherapies use immune cells. These include monoclonal antibody therapy and immune checkpoint targeting.³³ HCC cells have been shown to secrete CCL5, CCL22 and CCL28 chemokines to mediate the accumulation of Regulatory T cells, whereas the key immunosuppressive role of CD4⁺ CD25⁺ Foxp3 regulatory T cells in the tumour microenvironment has recently been reported as a potential target for new immunotherapies.^{34,35} Different chemokines have pro-tumour and anti-tumour functions because they mediate different immune cell migration processes. Chemokine networks have become potential immunotherapeutic targets.³ In our study, the low-risk group had higher immune-related function scores and expression of several immune checkpoint genes. They also had relatively higher immune cell scores in the TME score, suggesting that immunotherapy may be more effective in the low-risk group. In addition, we also verified that the gene model was used to predict the response of HCC patients to sorafenib treatment, and sorafenib treatment is more likely to benefit high-risk patients. Sorafenib and regrafenib, as tyrosine kinase inhibitors, play an important role in anti-tumour angiogenesis and inhibition of proliferation. Some studies have reported the activity of both drugs on normalisation of the vascular system and stimulation of anti-tumour immunity in HCC after combination therapy with immune checkpoint inhibitors. The combination therapy inhibited STAT3 activity, increased CXCL10 expression and prolonged the survival of activated CD8 T cells.^{36,37} So there are more CCRs that may be targets for HCC targeting and immunotherapy, which awaits our further research.

Conclusion

The CCRs-related gene signature may better assess HCC prognosis and response to immunotherapy and tyrosine kinase inhibitors such as sorafenib in HCC, providing prospective solutions for diagnosis and treatment.

Data Sharing Statement

This study analyses publicly available datasets, the names and links of which are given in the article.

Ethical Statement

This study was approved by the ethics committee of the Second Affiliated Hospital of Nanchang University.

Funding

This study was supported by the National Natural Science Foundation of China (82260570) and the Jiangxi Province Graduate Innovation Special Fund Project (YC2023-B088).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Dong G, Zhang S, Shen S, et al. SPATS2, negatively regulated by miR-145-5p, promotes hepatocellular carcinoma progression through regulating cell cycle. *Cell Death Dis.* 2020;11(10):837. doi:10.1038/s41419-020-03039-y
2. Zhang FP, Huang YP, Luo WX, et al. Construction of a risk score prognosis model based on hepatocellular carcinoma microenvironment. *World J Gastroenterol.* 2020;26(2):134–153. doi:10.3748/wjg.v26.i2.134
3. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol.* 2017;17(9):559–572. doi:10.1038/nri.2017.49
4. Sanchez J, Lane JR, Canals M, Stone MJ. Influence of chemokine n-terminal modification on biased agonism at the chemokine receptor CCR1. *Int J Mol Sci.* 2019;20(10):2417. doi:10.3390/ijms20102417
5. Vinader V, Afarinkia K. A beginner's guide to chemokines. *Future Med Chem.* 2012;4(7):845–852. doi:10.4155/fmc.12.49
6. Schall TJ, Proudfoot AE. Overcoming hurdles in developing successful drugs targeting chemokine receptors. *Nat Rev Immunol.* 2011;11(5):355–363. doi:10.1038/nri2972
7. Sakai N, Yoshidome H, Shida T, et al. CXCR4/CXCL12 expression profile is associated with tumor microenvironment and clinical outcome of liver metastases of colorectal cancer. *Clin Exp Metastasis.* 2012;29(2):101–110. doi:10.1007/s10585-011-9433-5
8. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet.* 2001;357(9255):539–545. doi:10.1016/S0140-6736(00)04046-0
9. Lim SY, Yuzhalin AE, Gordon-Weeks AN, Muschel RJ. Targeting the CCL2-CCR2 signaling axis in cancer metastasis. *Oncotarget.* 2016;7(19):28697–28710. doi:10.18632/oncotarget.7376
10. Li L, Xu L, Yan J, et al. CXCR2-CXCL1 axis is correlated with neutrophil infiltration and predicts a poor prognosis in hepatocellular carcinoma. *J Exp Clin Cancer Res.* 2015;34:129. doi:10.1186/s13046-015-0247-1
11. Granito A, Forgione A, Marinelli S, et al. Experience with regorafenib in the treatment of hepatocellular carcinoma. *Therap Adv Gastroenterol.* 2021;14:17562848211016959. doi:10.1177/17562848211016959
12. Sasaki R, Kanda T, Fujisawa M, et al. Different mechanisms of action of regorafenib and lenvatinib on toll-like receptor-signaling pathways in human hepatoma cell lines. *Int J Mol Sci.* 2020;21(9):3349. doi:10.3390/ijms21093349
13. Debes JD, Romagnoli PA, Prieto J, et al. Serum biomarkers for the prediction of hepatocellular carcinoma. *Cancers.* 2021;13(7). doi:10.3390/cancers13071681
14. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. *Annu Rev Immunol.* 2014;32:659–702. doi:10.1146/annurev-immunol-032713-120145
15. Chen X, Chen R, Jin R, Huang Z. The role of CXCL chemokine family in the development and progression of gastric cancer. *Int J Clin Exp Pathol.* 2020;13(3):484–492.
16. Raza S, Rajak S, Tewari A, et al. Multifaceted role of chemokines in solid tumors: from biology to therapy. *Semin Cancer Biol.* 2022;86(Pt 3):1105–1121. doi:10.1016/j.semcancer.2021.12.011
17. Han KQ, He XQ, Ma MY, et al. Inflammatory microenvironment and expression of chemokines in hepatocellular carcinoma. *World J Gastroenterol.* 2015;21(16):4864–4874. doi:10.3748/wjg.v21.i16.4864
18. Marsigliante S, Vetrugno C, Muscella A. CCL20 induces migration and proliferation on breast epithelial cells. *J Cell Physiol.* 2013;228(9):1873–1883. doi:10.1002/jcp.24349
19. Frick VO, Rubie C, Keilholz U, Ghadjar P. Chemokine/chemokine receptor pair CCL20/CCR6 in human colorectal malignancy: an overview. *World J Gastroenterol.* 2016;22(2):833–841. doi:10.3748/wjg.v22.i2.833
20. Benkheil M, Van Haele M, Roskams T, et al. CCL20, a direct-acting pro-angiogenic chemokine induced by hepatitis C virus (HCV): potential role in HCV-related liver cancer. *Exp Cell Res.* 2018;372(2):168–177. doi:10.1016/j.yexcr.2018.09.023
21. Du D, Liu Y, Qian H, et al. The effects of the CCR6/CCL20 biological axis on the invasion and metastasis of hepatocellular carcinoma. *Int J Mol Sci.* 2014;15(4):6441–6452. doi:10.3390/ijms15046441
22. Ghadjar P, Rubie C, Aebersold DM, Keilholz U. The chemokine CCL20 and its receptor CCR6 in human malignancy with focus on colorectal cancer. *Int J Cancer.* 2009;125(4):741–745. doi:10.1002/ijc.24468
23. Bonaventura A, Montecucco F. CCL23 is a promising biomarker of injury in patients with ischaemic stroke. *J Intern Med.* 2018;283(5):476–478. doi:10.1111/joim.12742
24. Mayado A, Teodosio C, Garcia-Montero AC, et al. Increased IL6 plasma levels in indolent systemic mastocytosis patients are associated with high risk of disease progression. *Leukemia.* 2016;30(1):124–130. doi:10.1038/leu.2015.176
25. Karan D. CCL23 in balancing the act of endoplasmic reticulum stress and antitumor immunity in hepatocellular carcinoma. *Front Oncol.* 2021;11:727583. doi:10.3389/fonc.2021.727583
26. Jin L, Liu WR, Tian MX, et al. CCL24 contributes to HCC malignancy via RhoB- VEGFA-VEGFR2 angiogenesis pathway and indicates poor prognosis. *Oncotarget.* 2017;8(3):5135–5148. doi:10.18632/oncotarget.14095
27. Korbecki J, Kojder K, Simińska D, et al. CC chemokines in a tumor: a review of pro-cancer and anti-cancer properties of the ligands of receptors CCR1, CCR2, CCR3, and CCR4. *Int J Mol Sci.* 2020;21(21):1.
28. Xiong N, Fu Y, Hu S, Xia M, Yang J. CCR10 and its ligands in regulation of epithelial immunity and diseases. *Protein and Cell.* 2012;3(8):571–580. doi:10.1007/s13238-012-2927-3
29. Lazarus NH, Kunkel EJ, Johnston B, Wilson E, Youngman KR, Butcher EC. A common mucosal chemokine (mucosae-associated epithelial chemokine/CCL28) selectively attracts IgA plasmablasts. *J Immunol.* 2003;170(7):3799–3805. doi:10.4049/jimmunol.170.7.3799
30. Mergia Terefe E, Catalan Opulencia MJ, Rakhshani A, et al. Roles of CCR10/CCL27-CCL28 axis in tumour development: mechanisms, diagnostic and therapeutic approaches, and perspectives. *Expert Rev Mol Med.* 2022;24:e37.
31. Zhou Y, Zhang BH, Yin X, Ren ZG. Role of chemokine CCL28 in hypoxia-induced migration of hepatocellular carcinoma. *Zhonghua Gan Zang Bing Za Zhi.* 2013;21(7):524–527. doi:10.3760/cma.j.issn.1007-3418.2013.07.012
32. Ondondo B, Colbeck E, Jones E, et al. A distinct chemokine axis does not account for enrichment of Foxp3(+)CD4(+) T cells in carcinogen-induced fibrosarcomas. *Immunology.* 2015;145(1):94–104. doi:10.1111/imm.12430
33. Mollica Poeta V, Massara M, Capucetti A, Bonocchi R. Chemokines and chemokine receptors: new targets for cancer immunotherapy. *Front Immunol.* 2019;10:379. doi:10.3389/fimmu.2019.00379

34. Granito A, Muratori L, Lalanne C, et al. Hepatocellular carcinoma in viral and autoimmune liver diseases: role of CD4+ CD25+ Foxp3+ regulatory T cells in the immune microenvironment. *World J Gastroenterol.* 2021;27(22):2994–3009. doi:10.3748/wjg.v27.i22.2994
35. Ren L, Yu Y, Wang L, Zhu Z, Lu R, Yao Z. Hypoxia-induced CCL28 promotes recruitment of regulatory T cells and tumor growth in liver cancer. *Oncotarget.* 2016;7(46):75763–75773. doi:10.18632/oncotarget.12409
36. Stefanini B, Ielasi L, Chen R, et al. TKIs in combination with immunotherapy for hepatocellular carcinoma. *Expert Rev Anticancer Ther.* 2023;23(3):279–291. doi:10.1080/14737140.2023.2181162
37. Shigeta K, Matsui A, Kikuchi H, et al. Regorafenib combined with PD1 blockade increases CD8 T-cell infiltration by inducing CXCL10 expression in hepatocellular carcinoma. *J Immuno Thera Canc.* 2020;8(2):e001435. doi:10.1136/jitc-2020-001435

Cancer Management and Research

Dovepress

Publish your work in this journal

Cancer Management and Research is an international, peer-reviewed open access journal focusing on cancer research and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/cancer-management-and-research-journal>